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In the application of:

Suresh K. TIKOO, et al

Serial No.: 09/871,212

Filing Date: May 31, 2001

For: MODIFIED BOVINE ADENOVIRUS  
HAVING ALTERED TROPISM

Examiner: U. Winkler

Group Art Unit: 1648

**DECLARATION OF SURESH K. TIKOO  
PURSUANT TO 37 C.F.R § 1.132**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Dear Sir:

I, Suresh K. Tikoo, declare as follows:

1. I am currently Head, Vectored Vaccine Program of Vaccine and Infectious Disease Organization at the University of Saskatchewan. My Curriculum Vitae is attached hereto as Exhibit 1.
2. I currently reside at 302-102 Edinburgh Place, Saskatoon, Saskatchewan, Canada S7H 5J7.
3. I am an expert in the field of molecular biology, immunology and adenovirus technology. I am an inventor of the above-identified patent application and have reviewed the pending claims. I have also read the Office Action dated December 10, 2002.
4. Adenovirus capsid proteins are well known by those in the field of adenovirus technology and include hexon, penton and fiber proteins as well as proteins IIIa, VI, VIII and IX.

See Fields et al. *Fundamental Virology* (1991, 2nd edition, Raven Press, New York, pages 771-776); Colby et al. (1981, *J. Virol.* 39:977-980); and Akalu et al. (1999, *J. Virol.* 73:6182-6187).

5. I constructed a replication-competent bovine adenovirus 3 (BAV-3) comprising a modified capsid pIX gene comprising nucleic acid encoding an Arg-Gly-Asp (RGD) peptide. RGD motifs present on adenovirus penton proteins are thought to mediate viral attachment through interactions between the penton and integrins present on cells. See Mathias et al. (1994, *J. Virol.* 68:6811) and Vigne et al. (1999, *J. Virol.* 73:5156).

The overlapping synthetic oligonucleotides used to make a DNA sequence containing the RGD peptide are as shown below. Sense oligo sequence:

GGATCAGGATCAGGTTTCAGGGAGTGGCTCTCGCCTGCGACTGTCGCGGCGATTGTTT  
TTGCGGTTAAGTT and antisense:

AACTTAACCGCAAAAACAATCGCCGCGACAGTCGCAGGCAGAGCCACTCCCTGAAC  
CTGATCCTGATCC.

The oligonucleotides were mixed together and cloned into a plasmid comprising BAV3 nucleotides 1 to 10051 based on the BAV3 numbering shown in GenBank Accession Number AF030154. The resulting plasmid was named pBNdARGD. A fragment of the BAV-3 genome was inserted into pBNdARGD to extend homologous sequences for recombination in E.coli. Recombination was carried out in the E.coli strain BJ5183, which is publicly available, by routine methods and the resulting BAV-3 plasmid comprising the modified bovine adenovirus capsid pIX gene comprising nucleic acid encoding the RGD peptide was named pBAV950. The product length of pBAV950 increased to 1456 base pairs (bp) from the BAV-3 wild-type length of 1393 bp as measured by PCR using the following primers for analysis: P91 5' CTAATCGATACATGTACTACTG 3' (3057 bp of BAV-3 genome) and P92 5'CCAACCGGTTGTGGAAAATC 3'(4450 bp of BAV-3 genome).

Plasmid pBAV950 and wild-type BAV-3 were purified by ultracentrifugation in a gradient of CsCl. The proteins of purified virions were separated on 12% denaturing PAGE and analyzed in Western blotting using rabbit polyclonal anti-sera against BAV-3 pIX. Anti-pIX sera recognized a protein of 14 kDa in the wild-type BAV-3 virus and a 16 kDa protein in the recombinant BAV950. The sequence of the modified capsid pIX gene comprising nucleic acid encoding the RGD peptide is attached hereto as Exhibit 2. The genome of pBAV950 was demonstrated to be stable.

6. To evaluate whether pBAV950 results in modification of the BAV-3 tropism upon infection of human cells, the integrin-containing human cells, HeLa cells and A549 cells were infected with wild-type BAV-3 or pBAV950 at Multiplicity of Infection (m.o.i.) 100 TCID<sub>50</sub>/cell. After 2 hours of adsorption, the HeLa cells and A549 cells were washed twice with PBS and media was changed to MEM+10% FBS. At 48 hours after infection, cells were trypsinized and harvested. Total DNA was extracted from the cells, using QIAGEN DNAeasy Tissue Kit. Aliquots of 70 ng of total DNA were used in the Real Time PCR analysis. The primers were from the BAV-3 hexon gene sequence: RTP-1 TACAGTAATGTGGCGTTGTA and RTP-2 CGTATCAATAAGGCCGCTAA. The 5'-end labeled FAM (6-carboxy-fluorescein, reporter dye) and 3'-labeled TAMRA (6-carboxytetramethyl-rhodamine, quencher dye) probe was used in the PCR reaction. The sequence of the probe was CCGCCTAACCACGAACACCTACG. Dilutions of pBAV3 DNA (wild-type BAV-3) were used for absolute quantification of viral genomes in the DNA sample. Ten-fold more viral DNA was found in the BAV950 infected human HeLa and A549 cells, as compared to HeLa and A549 cells infected with wild type BAV-3.

7. The results demonstrate that BAV950, a BAV-3 having a modified capsid gene comprising nucleic acid encoding an RGD peptide, exhibits modified tropism for human cells.

8. The present patent application describes a bovine adenovirus comprising a modification in the fiber protein, designated BAV600. As described in the patent application, BAV600 comprises nucleic acid encoding a HAV-5 fiber knob region fused to the BAV-3 tail and shaft and further comprises nucleic acid encoding green fluorescence protein in the E3 region. Experiments disclosed in the examples of the present patent application demonstrate that BAV600 exhibits modified tropism and is able to transduce human HeLa cells, Hep-2 cells, 293 cells and A549 cells.

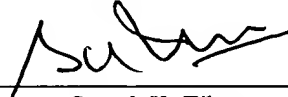
9. Following methods for transduction disclosed in the present specification in Example 3, BAV600 was used to infect swine testes cells (ST), Madin Darby canine kidney cells (MDCK), Crandell Ress kidney feline cells (CRKF), and African green monkey kidney cells (VERO). All the cell lines were obtained from public sources. The cells were grown in T25 flasks and infected with BAV600 at a m.o.i of 5. Forty eight hours after infection, the percentage of GFP-fluorescence positive cells were determined by flow cytometry. The results are attached hereto as Exhibit 3. Unexpectedly, bovine adenovirus BAV600 was able to

transduce porcine cells, canine cells, feline cells and African green monkey cells at a significantly higher level than control bovine adenovirus lacking the human fiber knob region.

10. The results demonstrate that BAV600, a BAV-3 comprising nucleic acid that encodes a HAV-5 fiber knob region fused to the BAV-3 tail and shaft, has altered tropism for non-human mammalian cells including porcine, canine, feline and African green monkey cells.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

June 6th, 2003  
Date

  
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Suresh K. Tikoo